



Foraging behavior of two egg parasitoids exploiting chemical cues from the stink bug *Piezodorus guildinii* (Hemiptera: Pentatomidae)

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Abstract: Several parasitoids attacking the same host may lead to competition. Adult parasitoids' abilities to find, parasitize and defend hosts determine resource's retention potential. In soybean, two egg parasitoid species, *Telenomus podisi* and *Trissolcus urichi* (Hymenoptera: Platygasteridae), compete on the egg masses of *Piezodorus guildinii* (Hemiptera: Pentatomidae) one of the major pest of this crop. We evaluated parasitoid's abilities to exploit hosts' footprints; and parasitoid's behavior when competing for the same host. Both arena residence time and retention time were similar for *T. podisi* and *T. urichi* on male or female host footprints. In its turn, *T. urichi* reentered the area contaminated with *P. guildinii* more times and staid longer in it than *T. podisi*. Furthermore, when competing for the same egg mass, each parasitoid species won (was in possession of the host by the end of the experiment) half of the replicates, and the number of times each wasp species contacted host in the first place was similar, without affecting replicate outcome (who ultimately won). Both species started agonistic and non-agonistic encounters. This study provides information about the potential interspecific competition between these parasitoids, which contributes to evaluate the compatibility of multiple natural enemies' biological control programs for stink bugs.

Key words: biological control, coexistence, natural enemies, Platygasteridae, searching behavior, stink bugs.

INTRODUCTION

Competition is an interaction that occurs among two or more individuals of the same or different species (intraspecific or interspecific, respectively), which arises when the supply of a scarce resource does not satisfy the immediate needs of individuals.

Insect parasitoids have a very specialized life cycle that includes an immature stage that develops on or within a single host, ultimately killing that host. Therefore, competition is considered a more important phenomenon in solitary parasitoids than at other trophic levels, since individual hosts constitute a small resource sufficient for only one parasitoid (Godfray et al. 1994). In nature, a single host species can have multiple parasitoid species, which often results in intense interactive

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competition for the host resource (Cusumano et al. 2016). Competition can occur between adult parasitoids (extrinsic competition), i.e. interactions between females searching for or exploiting hosts, as well as between immature parasitoids (intrinsic competition), i.e. between larvae developing in the same host (Zwölfer 1971). Zwölfer (1971) classified parasitoids as “extrinsically superior” if they have better skills in host location and “intrinsically superior” if they have better skills in larval competition. He argued that the superior dispersal capabilities of poorer intrinsic competitors are important in explaining coexistence (Fugitive species hypothesis).

In extrinsic competition, a parasitoids' ability to find a host, to parasitize it and to defend it determine the potential for retention of parasitoid's resource (Cusumano et al. 2016). Female parasitoids searching for hosts find and explore a variety of stimuli, among which chemical cues (semiochemical) play an important role (Conti and Colazza 2012, Fatouros et al. 2016). These cues may originate directly from the host or from their products such as faeces, silk, or exuviae. Additionally, they may be emitted from the food plants of hosts (Steidle and van Loon 2003). Cues not produced directly from the host may be originated far from the exact location of it. The localization and recognition of suitable hosts is a complex process, especially in oophagous parasitoids, since eggs are generally available for a short period of time given their rapid development. As semiochemicals coming from egg masses may be undetectable, oophagous parasitoids have developed specialized searching strategies. Several species of hymenopteran oophagous parasitoids from the family Platygasteridae use footprints left by adults and/or juveniles of their hosts, and the typical response to these cues is a retention behavior (the parasitoid remains in the vicinity) (Colazza et al. 2014). The ability to discriminate semiochemicals

originated from gravid females could ensure parasitoids location of newly laid eggs.

Pentatomids are the main soybean seed sucking pests. From them, *Piezodorus guildinii* (Westwood) (Hemiptera: Pentatomidae) is the most important species in Argentina (Gamundi and Sosa 2008). It is also one of the most relevant stink bugs in Brazil (Corrêa-Ferreira 2008), Uruguay (Castiglioni et al. 2008) and several states of the United States (Temple et al. 2013). From the whole complex of soybean stink bugs, *P. guildinii* provokes the deepest seed damage (Depieri and Panizzi 2011) and causes greater leaf retention causing problems in the harvesting process (Corrêa-Ferreira and Azevedo 2002). Moreover, *P. guildinii* is highly mobile and more difficult to control with insecticides (Temple 2011). Adults are not attacked by parasitoids (Liljesthröm and Avalos 2015), as happens with the other stink bug species in the complex, what represents an empty niche. Egg parasitoids are important natural enemies that limit stink bug numerical increase. These parasitoids are considered “quasi-gregarious” due to the oviposition habits of their hosts, who lay their eggs in patches (egg masses). Concerning *P. guildinii* egg parasitoids, in the Neotropical region the most common species are *Telenomus podisi* (Ashmead) and *Trissolcus urichi* (Crawford) (Hymenoptera: Platygasteridae).

Evidences of competition among different parasitoids species have been documented from host egg masses parasitized in field from which two or more different parasitoids species have emerged from individual eggs (Cusumano et al. 2011). For example, *T. podisi* and *T. urichi* or *Trissolcus basalis* Wollaston (Hymenoptera: Pentatomidae) emerged together from different eggs of the same *P. guildinii* egg mass (Cingolani et al. 2014a).

Cingolani et al. (2013) found that neither *T. podisi* nor *T. urichi* avoided multiparasitism when in indirect (exploitative) competition, however, the outcome of multiparasitism was less favorable

for *T. podisi* irrespective of whether it was the first or second to parasitize. The number of *T. podisi* adults emerging was significantly lower than for *T. urichi*. Furthermore, the proportion of *T. podisi* emerging from multiparasitized eggs was lower than the proportion emerging from eggs parasitized by this species only. Therefore, *T. urichi* seems to be a better intrinsic competitor and potentially able to exclude *T. podisi*. In the field, however, *T. podisi* was always the dominant species on *P. guildinii* egg masses whereas *T. urichi* was recorded only when host abundance was high (Cingolani et al. 2014a).

Several authors have investigated the performance of platygastriids on different hosts under laboratory conditions (Powell and Shepard 1982, Liljesthröm and Cameán 1992, Corrêa-Ferreira 1993, Colazza and Bin 1995, Pacheco and Corrêa-Ferreira 1998, Kivan and Kiliç 2002, Sujii et al. 2002), although very few have evaluated *P. guildinii* as a host (Cingolani et al. 2014b).

Understanding how interspecific competition between parasitoids can affect pest suppression may help to improve biological control. Research in this area has focused mainly on intrinsic competition while it is largely unexplored how extrinsic competition can affect biological control (Cusumano et al. 2012a). Although it is relatively straightforward to determine intrinsic competitive superiority by examining the outcome of multiparasitism, it is far harder to demonstrate extrinsic competitive superiority. Experimental studies of comparative host location are needed to assess extrinsic competitive superiority (Cusumano et al. 2012a). Regarding species of our study, the coexistence of *T. urichi* and *T. podisi* in soybean fields could be favored by compensation of skills in larval competition and host location efficiency of both parasitoid species. The specific objectives of our work were: 1) to evaluate the comparative abilities of *T. podisi* and *T. urichi* to locate and pursuit footprints left by male and female *P.*

guildinii; 2) to evaluate *T. podisi* and *T. urichi* behavior when exploiting *P. guildinii* egg masses.

MATERIALS AND METHODS

INSECT COLONIES

We established stink bug and wasp cultures from individuals collected in Argentinean soybean fields during the 2015-2016 crop growing season. Adults of *P. guildinii* were kept in plastic cages 20x20x20 cm with a ventilation window under laboratory conditions ($25\pm1^{\circ}\text{C}$; $70\pm10\%\text{RH}$). We provided fresh *Phaseolus vulgaris* (L.) (Fabales: Fabaceae) pods twice a week for feeding. Eggs were collected daily and preserved under the same conditions, for stink bugs and wasps colonies maintenance. Parasitoids (*T. podisi* and *T. urichi*) were reared on eggs of this stink bug and honey was provided daily as food source for adults.

The day before wasp females were used in experiments, they were isolated from the colony and placed in test tubes with honey. Female wasps were naïve to oviposition experience and host chemicals, were mated, and were 2-4 days old when used in trials. Wasps were allowed to acclimate in the experiment room 3 h before bioassays. Tests were conducted from 9:00 am to 1:00 pm in an isolated room under laboratory conditions.

We performed two different experiments: (1) we exposed parasitoids to chemical cues not directly associated with the presence of host eggs (host traces bioassays), and (2) we exposed parasitoids directly to a host egg mass (host egg masses bioassays).

HOST TRACES BIOASSAYS

Parasitoids' response to adult host footprints was investigated in an open arena consisting of a square sheet of filter paper (25x25 cm). In the center of the arena, a circular area (6 cm diameter) was demarcated in the underside of the filter paper using a pencil, and this area was exposed for 30

min to the stimuli, leaving the surrounding area of the arena untreated. The demarcation of the circular area was visualized by translucency. Each arena was used for testing only one wasp female. The stimuli (treatments) were: (1) footprints left by *P. guildinii* males, (2) footprints left by *P. guildinii* mated females in preoviposition state, (3) and a negative control, i.e. the same arena but without stink bug's footprints. For treatments 1 and 2, the exposition to stimuli was performed by keeping an adult of stink bug walking on the filter paper and let it to move continuously and uniformly in the constrained area. To ensure a constant contact of the bug legs onto the filter paper and, at the same time, to avoid contamination of the surface of the filter paper with possible bug volatiles, adults were constrained under a steel mesh cover, following Peri et al. (2006) methodology. Open arenas contaminated by bug's feces were not used for bioassays. *Telenomus podisi* and *T. urichi* female wasps were tested singly by gentle release in the middle of the circular area, and each wasp was tested only once. We performed 30 replicates for each treatment. Parasitoid behavior was visually observed until wasps flew away from or walked off the whole arena (the filter paper). We registered the time spent by wasps in each part of the arena (i.e. inside and outside the central circular area) using a stopwatch, and the number of times the wasp reentered this circular area (in case she had previously abandoned it).

Wasps' behavior was evaluated in terms of: (1) arena residence time, quantified over the entire arena pooling times spent by wasps in both inside and outside the central circular area, (2) retention time, i.e. the time spent by wasps inside the central circular area, (3) number of times each wasp reentered the central circular area.

Arena residence time was analyzed with a Kruskal-Wallis test. The proportion of the replicate total time that each wasp spent inside the central area was also compared with a Kruskal-Wallis test.

In all cases, comparisons of the average ranks for each pair of combinations were made computing normal z-values, as well as post hoc probabilities for a two sided test of significance (Zar 1996). We also compared residence time, inside ("retention time") or outside the treated area by evaluating Kaplan–Meier estimators and different Kaplan–Meier curves were compared by the log rank test. Additionally, the proportion of replicates in which each wasp reentered the central circular area was statistically compared with a Fisher exact test.

HOST EGG MASSES BIOASSAY

We studied behavioral interspecific interactions between adult platygastriids when searching for hosts simultaneously. One *T. podisi* and one *T. urichi* female wasp were released at the same moment into a small Petri dish (5 cm in diameter) containing an average host egg mass (15 eggs/egg mass). Eggs were less than 24 h old (the preferred development stage of the host, for these oophagous parasitoids), and were added to the arena using forceps. Behaviors of both females were observed for 2 h, and two types of encounters between females were distinguished. The first type was non-agonistic encounters, in which females either physically contacted one another or came into extremely close proximity but exhibited no aggression. The second type was agonistic encounters, pooling both backdowns (one individual attacking and the other retreating without retaliation) and escalated fights (one individual attacking and the other retaliating and initiating a bout of mutual aggression). The female that possessed the host by the end of the experiment was assumed as the winner, whether there have been direct interactions between wasps or not during the observation period. We performed 22 replicates, and individual females and host egg masses were used in experiments only once.

We analyzed the proportion of replicates in which each wasp species won the host egg mass

performing a Fisher exact test. We also analyzed the proportion of replicates in which each wasp species was the first one in contacting the host egg mass performing a Fisher exact test, and analyzed the effect of being the first or the second in contacting the egg mass, on replicate's outcome (who ultimately won or lost the egg mass) with a contingency table. Finally, we analyzed the number of agonistic and of non-agonistic encounters between *T. podisi* and *T. urichi* females searching for a host egg mass with ANOVA. Analyses were performed using R software (R Core Team 2014).

RESULTS

HOST TRACES BIOASSAYS

When analyzing the arena residence time (time spent by wasps in the entire arena) wasps that were exposed to control treatment remained significantly less time on the arena than wasps exposed to host traces, regardless wasp species or host gender. On average, both *T. podisi* and *T. urichi* staid just about half a minute exploiting the arena in the control treatment before leaving ($H_{(5;N=180)}=114.622$; $p<0.001$). Nonetheless, we removed control treatment from analysis and there were no differences in the arena residence time of *T. podisi* and *T. urichi* towards stink bug traces generated by female or male hosts ($H_{(3;N=120)}=6.206$; $p<0.102$) (Figure 1).

When comparing wasps' retention time (time spent by wasps inside the central circular area), both wasp species spent less time on areas without host traces (control) than on areas contaminated with host footprints ($\chi^2=125.27$; $df=5$; $p<0.001$). However, we did not found differences in *T. podisi* or *T. urichi* retention time towards female or male hosts traces ($\chi^2=1.93$; $df=3$; $p=0.586$) when analysis was made without considering data from control treatment (Table I).

When comparing time spent by wasps outside the central circular area, both wasp species spent

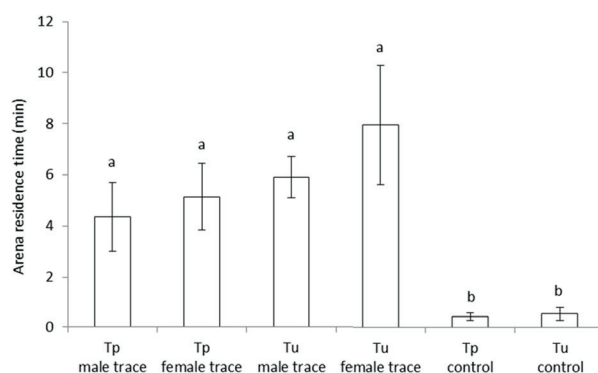


Figure 1 - Arena residence time (average time spent by wasps in the entire arena) when exploiting traces from *Piezodorus guildinii* female or male, or no stink bug traces (control). Different letters indicate significant differences. Bars denote 95% confidence intervals. Tp: *Telenomus podisi*; Tu: *Trissolcus urichi*.

less time in control treatment than in male or female host traces treatments ($\chi^2=116.051$; $df=5$; $p<0.001$). Moreover, when we removed control treatment from analysis, we found differences between wasp species ($\chi^2=17.2$; $df=3$; $p<0.001$), denoting that the risk of *T. urichi* abandoning the not treated area doubles the risk of *T. podisi* abandoning it ($p<0.001$).

From total replicate duration, *T. urichi* spent a higher proportion of time inside the central area treated with stink bug footprints (in average, 0.857 on male host traces and 0.845 on female host traces), and just a little proportion of time outside the central area where there were no host footprints. On the contrary, *T. podisi* spent a similar proportion of time inside the central area treated with female or male hosts (in average, 0.575 on female host traces and 0.479 on male host traces), and outside it. The same situation was seen in wasps from both species exposed to control treatment. The proportion of time of total replicate duration that *T. urichi* invested in exploiting stink bug's traces was significantly higher than the proportion of time invested by *T. podisi* on the circular treated area and the time invested by both wasp species on the

TABLE I

Average time (min \pm SD) spent by parasitoid wasps (*T. podisi* and *T. urichi*) inside and outside the central circular area, treated with ♀ or ♂ *P. guildinii* (host) traces, or not exposed to host traces (control).

	Control		Host traces			
	♀		♂			
	inside	outside	inside	outside	inside	outside
<i>T. podisi</i>	0.189 \pm 0.158 ^B	0.237 \pm 0.363 ^c	3.593 \pm 3.523 ^A	1.552 \pm 1.296 ^a	2.648 \pm 3.491 ^A	1.712 \pm 2.340 ^a
<i>T. urichi</i>	0.371 \pm 0.590 ^B	0.177 \pm 0.210 ^c	5.675 \pm 1.786 ^A	1.157 \pm 0.932 ^b	5.179 \pm 2.123 ^A	0.747 \pm 0.484 ^b

Different letters indicate significant differences in time spent by wasps inside (uppercase) or outside (lowercase) the central circular area, among treatments.

central area in control treatments ($H_{(5;N=180)}=34.264$; $p<0.001$).

When analyzing the number of times *T. podisi* and *T. urichi* reentered the central circular area, we found that on control treatments wasps almost never reentered the central area after leaving (it happened only in 2 out of 30 replicates from *T. urichi*). From treatments where the central circular area was exposed to stink bugs' footprints, *T. podisi* usually reentered the treated area less often than *T. urichi*. *Telenomus podisi* reentered the area treated with male host's traces only in 8 of the 30 replicates, and in the remaining 22 replicates the wasps abandoned the whole arena upon leaving the treated area and never returned. On the contrary, *T. urichi* abandoned the treated area and then reentered it in almost all the 30 replicates (29 out of 30), in fact reentering numerous times in each replicate. Similar results were found when wasps exploited female host traces (*T. podisi* reentered in 12 out of 30 replicates; *T. urichi* reentered in 29 out of 30 replicates). The proportion of times *T. urichi* reentered the area contaminated with female or male *P. guildinii* was higher than the proportion for *T. podisi* reentering area contaminated with female (Fisher exact test $p<0.001$) or male hosts (Fisher exact test $p<0.001$). On average, *T. podisi* reentered the central area with stink bug's footprints 1.5 times per replicate, both for male or female traces. In contrast, *T. urichi* exploited host traces more insistently, reentering on average 4.43 and 5.47

times per replicate from male and female traces, respectively.

HOST EGG MASSES BIOASSAY

Each parasitoid species won about half of the replicates (*T. podisi* won 8 times; *T. urichi* won 12 times; 2 of the replicates ended tied) (Fisher exact test $p=0.214$). The number of times wasps from either species contacted the host egg mass in the first place was similar (*T. podisi*: 12; *T. urichi*: 10) (Fisher exact test $p=0.554$), and it did not affect replicate resolution ($\chi^2=0.952$; $df=1$; $p=0.329$) as *T. podisi* won 6 times being the first who have contacted the egg mass, and *T. urichi* won 8 times being the first who have contacted the egg mass. In four of the replicates *T. podisi* was the species who contacted the egg mass first but she was displaced by a female of *T. urichi* out from the egg mass, forcing *T. podisi* to lose the host. A similar situation occurred in two of the replicate where *T. urichi* was first to contact the egg mass but *T. podisi* finally won the host.

Both parasitoid species started non-agonistic encounters in a similar number of times ($F=0.536$; $df=1$; $p=0.468$) (Figure 2). When encountering a heterospecific competitor during host exploitation, *T. podisi* and *T. urichi* females displayed agonistic behaviors. The repertoire of agonistic behaviors consisted of chasing and hitting, behaviors that led to the departure of one of the females (the loser) from the host egg mass. The number of times each species started agonistic encounters was also

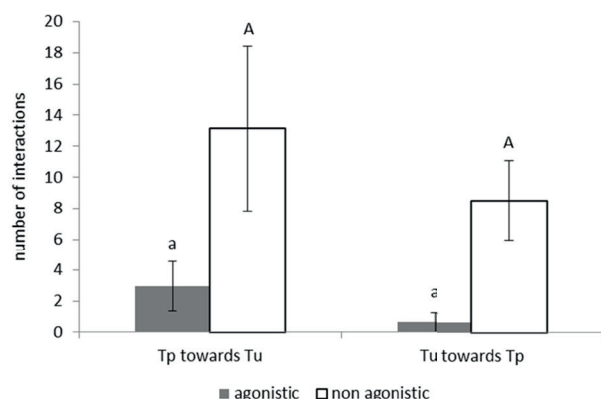


Figure 2 - Mean agonistic and non-agonistic encounters per trial between *Telenomus podisi* (Tp) and *Trissolcus urichi* (Tu) when searching simultaneously for *Piezodorus guildinii* egg masses. Different letters indicate significant differences in the number of agonistic (small letters) or non-agonistic (capital letters) encounters between wasps. Bars denote 95% confidence intervals.

similar for *T. podisi* and *T. urichi* ($F=1.593$; $df=2$; $p=0.214$) (Figure 2). Thereby, *T. podisi* seemed to be more aggressive than *T. urichi*, attacking more insistently. During all of these observations no females were killed, but if well injuries were rare, in two cases *T. urichi* females ended with the distal segment of two or three of their legs bitten off. In any case, this did not appear to impair the female's ability to continue searching for the host.

DISCUSSION

We found that *T. urichi* was more insistent than *T. podisi* when exploiting *P. guildinii* footprints, searching more actively and spending significantly more time on host traces. These results demonstrate some difference in the relative response of parasitoid species to kairomones, which can affect the success of the parasitoid that exploits hosts' cues while searching for eggs.

Lo Giudice et al. (2010) demonstrated the importance of plant surfaces in mediating interactions between chemical footprints of hosts and their egg parasitoids. They found that epicuticular waxes of leaves of broad bean, *Vicia faba* L. (Fabales: Fabaceae) and collard greens,

Brassica oleracea L. (Brassicales: Brassicaceae), mediate the foraging behavior of *T. basalis* by adsorbing contact kairomones from adults of *Nezara viridula* L. (Hemiptera: Pentatomidae). Although the experimental units we used for the trials are rather artificial in representing natural conditions found by wasps in field, both wasp species only remained a very short period of time searching on the control treatment. That may indicate that filter paper absorbed properly the contact kairomones from *P. guildinii*.

In field conditions, to find hosts, egg parasitoid females can exploit semiochemicals arising from the host eggs, from the interaction of the plant and the eggs, or from stages other than the one attacked (Powell and Wright 1992, Fatouros et al. 2008). This last group of semiochemicals (indirect host-related cues) brings female wasps in proximity to potential host eggs but does not provide information on the location of host eggs itself. When in contact with an area contaminated with semiochemicals wasps show an arrestment response (i.e. a motionless period with the antennae kept in contact with the surface) followed by an intense searching behavior characterized by variation in orthokinesis and klinokinesis, and increased turning frequency (Conti et al. 2004). If no host eggs are found, wasps resume the normal walking pattern, abandoning the contaminated area.

Colazza et al. (1999) and Peri et al. (2011) found that *T. basalis* is able to exploit volatile oviposition-induced synomones, volatiles cues from virgin males and preovipositing females, and contact kairomones in the host footprints, while *Ooencyrtus telenomicida* (Vassiliev) (Hymenoptera: Encyrtidae) seems to use volatiles from host virgin males and from host mated females in preovipositional state. They argue that this diversity in terms of the nature of cues exploited may explain the superior host finding abilities of *T. basalis*, evidenced by higher *T. basalis* parasitization rate recorded in the field.

Concerning species of our work, numerous studies have suggested that several compounds could influence the behavior of *T. podisi* (Borges et al. 1999, 2003, Blassioli Moraes et al. 2005, 2009) but little is known about the diversity of cues that *T. urichi* can exploit during host finding process.

It was demonstrated that several platygastriid species only discriminates between footprints left by females or male host, when traces were left by their associated hosts (Colazza et al. 1999, 2007, Conti et al. 2004, Salerno et al. 2009, Peri et al. 2013). Peri et al. (2013) evaluated the ability of three platygastriid egg parasitoid species to detect adult host sex on the basis of the host traces left by three pentatomid species on filter paper. They found that wasps discriminated between chemical traces left by a pentatomid female versus male, exhibiting a clear preference for female traces only when these belonged to their associated hosts. No significant differences were observed when wasps were in contact with footprints of non-associated species. The two wasp species evaluated in our study showed high parasitism proportions when *P. guildinii* eggs were offered as hosts in laboratory assays (Cingolani et al. 2014b). However *P. guildinii* may not be the chosen host if the wasps are allowed to choose among different pentatomid species eggs. The response of *T. podisi* and *T. urichi* to host sex traces may indicate that *P. guildinii* is not the preferred host for these parasitoid species.

Another possible explanation for our results could be a differential use of infochemicals by *T. podisi* and *T. urichi* in relation to their dietary specialization. According to this concept, the more specialized a parasitoid is, the more information it needs to know for locating a host. Therefore, egg parasitoid species with a narrow host range should exploit specific cues more frequently than generalist species. Generalists need to invest less time in searching particular host species than specialists. Therefore, the use of infochemicals in order to reduce searching time is less important.

When comparing *T. podisi* and *T. urichi* dietary specialization, the former is a generalist parasitoid that was reported from more than 20 different host species, but *T. urichi* is a more specialized parasitoid reported from only 10 different host species (Rider 2015). Additionally, as suggested by Margaría (2008), the Telenomini tribe is under adaptive radiation, so this process could be influencing wasps' behaviors and their host's preferences in the field. In our study, *T. urichi* dedicated a higher proportion of time to exploit host traces than *T. podisi*. This could indicate a differential use of infochemicals in relation of differences in these species' dietary specializations, and may be an explanation for the prevalence of *T. podisi* on field collected *P. guildinii* eggs (Cingolani et al. 2014a). Theory suggests that specialist species are more competitive in their optimal habitats. However, empirical work has shown that specialist species are declining worldwide due to habitat loss and fragmentation (Büchi and Vuilleumier 2014). This could be the case of these two parasitoid species that exploit a common host, *T. urichi* being more competitive in laboratory assays but *T. podisi* being more competitive in natural communities.

The females of a lot of parasitoid insect species are time limited, unable to find enough hosts in which to lay all their eggs in one searching period. Time spent searching a patch on which there are few suitable hosts could be considered wasteful, so that allocation of time spent searching a patch becomes an important factor in the reproductive success of a parasitoid female (Nelson and Roitberg 1995). Optimal foraging theory (Stephens and Krebs 1986) assumes that animals adjust their behavior to maximize their lifetime reproductive success under different ecological conditions. The resources used by female insect parasitoids are hosts that can be dispersed in the habitat or aggregated in patches, and whose value varies, for example, according to the host species, among other characteristics. How long, and therefore to what

limits, patches should be exploited is one of the key factors determining the foraging success of female parasitoids and is therefore a central question in the behavioral ecology of insect parasitoids. In this sense, Peri et al. (2006) indicated that females of *T. basalis* and *Trissolcus brochymenae* (Ashmead) (Hymenoptera: Platygasteridae) are able to learn the features of their foraging environment and to adjust accordingly the amount of time spent on the patches of kairomones they are visiting, depending on whether or not host eggs are found. Leaving earlier a patch containing indirect indicators of host presence, as semiochemicals originated from male hosts, enables parasitoids to forage another patch and therefore to increase parasitoid's opportunity to find host eggs.

When we exposed parasitoids directly to a host egg mass, we found no differences in *T. podisi* and *T. urichi* abilities to find and take possession of host eggs. Our experiments did not assess the effect of some characteristics that can influence the ease with which wasps find their hosts in the field, such as differences in dispersal abilities, host preference, learning abilities, etc., since we performed tests in small Petri dishes and we used young, naïve and fed wasps. This kind of experiments may provide some information about potential behavior of wasps, but more complicated semifield experiments should be performed to evidence differential host finding abilities between species.

Frequently, after one wasp species had oviposited, a female of the other species took over and started parasitizing another host egg without disturbing each other. In other cases, when encountering a heterospecific during host exploitation, both *T. podisi* and *T. urichi* females displayed agonistic behaviors, from non-aggressive contacts to full attacks, towards their opponent. Although both species committed a similar number of aggressive encounters one against the other, we observed that the intensity of these encounters were somewhat unbalanced. *Telenomus podisi* seemed

to be always more aggressive than *T. urichi*. Moreover, even only few escalations led to serious injuries, the perpetrator was always *T. podisi*. This is an observation we made while performing the experiments, however, we did not quantify this aggressiveness in order to search for statistically significant differences. We just counted the number of agonistic encounters without giving a dimension to the aggression.

If well we did not analyze the resultant outcome in terms of offspring production, which is most strongly linked to fitness, in most cases the emerged wasps were all of the species who had had greater possession of the egg mass. In only five of the replicates we registered emergence of wasps of both species from one egg mass, although in those cases the proportion of emerged *T. urichi* individuals was always greater than the proportion of *T. podisi* emerged individuals. This may be because of multiparasitism of some of the eggs of the egg mass, and to the known supremacy of *T. urichi* in the intrinsic competition (Cingolani et al. 2013).

Aggressive host and brood guarding behaviors have been documented for many other platygasterid species. For example, Field and Calbert (1999) reported that intruder-owner contests for host-patches in female *T. basalis* rarely result in escalation: more frequently, the owner attacks and the intruder retreats. While the majority of works concern intraspecific interactions (Field 1998, Field and Calbert 1998, Field et al. 1998), behavioral defense against allospecifics is also reported (Cusumano et al. 2012b, 2013, Peri et al. 2014).

Although we did not quantify how injurious and/or potentially fatal encounters were, examination of interspecific interactions revealed that while agonistic contests often occur, fatalities do not. Game-theoretic models predict that both contestants benefit from avoidance of injurious or fatal fighting when the contested resource has

lesser value to contestants than resources expected to be found in the future, in which case contests are likely to be settled by non-injurious mechanisms such as convention or display (Maynard Smith and Parker 1976, Enquist and Leimar 1990). Fights resulting in injury or death are only expected when the value of the contested resource is similar to or greater than the expected value of future resources (Enquist and Leimar 1990). In animal contests, theoretical studies using game theory modeling and the concept of evolutionarily stable strategies (Maynard Smith 1974) suggest that the competitive abilities of competitors are defined in terms of their resource-holding potential (i.e. difference in the abilities of the contestants to acquire and retain resources) (Maynard Smith and Parker 1976, Hammerstein 1981), with the contested resource not necessarily being of equal value to each competitor (Hughes 1979, Goubault et al. 2007). First-arriving individuals usually place a higher value on the resource and contests are predicted to be won by the contestant with the highest resource-holding potential and/or resource value (Enquist and Leimar 1987, Parker 1974). Even though we did not vary the conditions influencing the outcomes of contests, like subjective and objective resource value, as *T. podisi* is more generalist than *T. urichi*, *P. guildinii* eggs could have resulted of somewhat lesser value for the former parasitoid than for the latter.

Since for parasitoids there is a direct link between host encounter rate and the production of offspring, and considering that each parasitized host is killed, parasitoid searching efficiency and resource holding potential is also crucial to parasitoid-host population dynamics (Vet 2001). As most parasitoid hosts are utilized by more than one species of parasitoid, understanding the ecological factors that facilitate the coexistence of competing parasitoids and the factors that determine which species prevails is important both for basic ecology and for the applied science of

biological control (De Moraes and Mescher 2005). Our results offer some insight into the potential interspecific competition between *T. podisi* and *T. urichi*, in terms of their host exploitation behavior. This information may contribute to manage such interactions to achieve sustainable management of pest populations through biological control, and to evaluate the compatibility of the use of multiple natural enemies for stink bugs control programs.

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AUTHOR CONTRIBUTIONS

MFC contributed to the manuscript in terms of project design, data collection and analysis, results interpretation and paper writing. MCB contributed to the manuscript in terms of data collection, results interpretation and paper writing. GGL contributed to the manuscript in terms of data analysis, results interpretation and paper writing. SC contributed to the manuscript in terms of results interpretation and paper writing.

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